HFE gene mutations in susceptibility to childhood leukemia: HuGE review

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The hereditary hemochromatosis (HHC) gene, *HFE* on chromosome 6p21.3, encodes a protein involved in iron homeostasis. *HFE* mutations have low penetrance with a mild effect on serum iron levels. Animal, twin, and population studies have shown that carrier state for *C282Y* can increase iron levels. A proportion of heterozygotes show slightly elevated serum iron levels. Increased serum iron has been suggested to increase the risk for oxidative damage to DNA. Epidemiologic studies established a correlation between iron levels and cancer risk. Case-control studies have reported associations between *HFE* mutations *C282Y*/*H63D* and several cancers, some of which in interaction with the transferrin receptor gene *TFRC* or dietary iron intake. Increased cancer risk in *C282Y* carriers is likely due to higher iron levels in a multifactorial setting. In childhood acute lymphoblastic leukemia (ALL), there is an association of *C282Y* with a gender effect in two British populations. No association has been found in acute myeloblastic leukemia and Hodgkin disease in adults. The childhood leukemia association possibly results from elevated intracellular iron in lymphoid cells increasing the vulnerability to DNA damage at a critical time window during lymphoid cell development. Interactions of *HFE* with environmental and genetic factors, most of which are recognized, may play a role in modification of susceptibility to leukemia conferred by *C282Y*. Given the population frequency of *C282Y* and the connection between iron and cancer, clarification of the mechanism of *HFE* associations in leukemia and cancer will have strong implications in public health. ***Genet Med* 2005:7(3):159–168.**

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Hereditary hemochromatosis (HHC) is a common autosomal recessive iron overload disease (OMIM 235200).[1] Because iron accumulation in vital organs and subsequent damage takes a long time, the clinical onset is usually at or after middle age. Traditionally, the disease has been diagnosed by assessment of the biochemical iron parameters (serum iron and ferritin levels, transferrin saturation). The gene responsible for the majority of HHC cases has been identified as the *HFE* gene on chromosome 6p21.3.[2] The use of molecular testing in predictive diagnosis has been problematic because of the lack of strong phenotype-genotype correlation.[1,3–6]

Recent efforts have defined genetic heterogeneity for hereditary forms of iron overload and identified most of the genes responsible.[7,8] Besides autosomal recessive classic HHC, other forms of hereditary iron overload exist (Table 1). The features and molecular genetics of non-HFE hemochromatosis are reviewed elsewhere.[7–9] The genes responsible for African (OMIM 601195) and neonatal (OMIM 231100) iron overload are still unknown (neonatal iron overload may be an alloimmune condition rather than genetic[10]). The importance of the genetic heterogeneity is that it may have caused misclassification error and, subsequently, some of the discrepancies in phenotype-genotype correlation in earlier HHC research.

Low penetrance of *C282Y* in causation of clinical HHC has been established by linkage[30] and molecular studies[3–5,31–37] (see review[6]). Specifically, some population-based mass screenings have shown that $< 1\%$ of homozygotes develop frank clinical hemochromatosis.[35–37] It may be that expression of *C282Y* as a clinical disorder requires the participation of other genes or environmental factors.

GENE VARIANTS

Originally, two missense alterations were identified in the *HFE* gene that occur at high frequencies in HHC patients and in the general population: a G to an A at nucleotide 845 of the original mRNA sequence (GI:1469789) in the amino acid codon 282 in exon 4 (*C282Y*), and a C to a G at nucleotide 187 in the same sequence in the amino acid codon 63 in exon 2 (*H63D*). A third missense mutation in exon 2 (nucleotide 193

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Table 1
Genes involved in regulation of body iron content

Gene	Gene symbol (GeneID)	Map position	Disease (OMIM no.)	References
Hemochromatosis	*HFE* (3077)	6p21.3	Hereditary hemochromatosis (235200)	11
Hemojuvelin	*HFE2*[a] (148738)	1q21.2	Juvenile hemochromatosis A (HFE2A; 602390)	12,13
Hepcidin	*HAMP* (57817)	19q13.1	Juvenile hemochromatosis B (HFE2B; 606464)	14–17
Transferrin receptor-2	*TFR2* (7036)	7q22	Hemochromatosis type 3 (HFE3; 604250)	18
Solute carrier family 40 (Ferroportin 1)	*SCL40A1*[b] (30061)	2q32	Hemochromatosis type 4 (HFE4; 606069)	19,20
Ferritin heavy chain 1	*FTH1* (397030)	11q13	Familial iron overload (134770)	21
Transferrin	*TF* (7018)	3q22.1	See OMIM 190000[c,d]	22
Ceruloplasmin (Ferroxidase)	*CP* (1356)	3q23-q25	See OMIM 117700[c,e]	23
Transferrin receptor-1	*TFRC* (7037)	3q26.2-qter	See OMIM 190010	23
Haptoglobin	*HP* (3240)	16q22.1	See OMIM 140100	23

[a]This gene name does not conform to the conventions because hemojuvelin gene is not related to *HFE*; [b]formerly named *SLC11A3*; [c]atransferrinemia and aceruloplasminemia can cause iron overload; [d]the C2 variant (*P570S*) interacts with *C282Y* in Alzheimer disease susceptibility[24]; [e]*S142G* polymorphism interacts with *C282Y* in genetic associations with cancer susceptibility[25–27] but no effect on HHC.[28] Functionally important mutations of iron storage disease genes are reviewed elsewhere.[29]

in the mRNA sequence), *S65C*, has recently been identified that may contribute to the development of a mild form of HHC.[38,39] Adherence to the above nucleotide numbers in description of these mutations is common practice but conflicts with the current principles of nomenclature. Acceptable unequivocal description of these three *HFE* mutations is shown in Table 2.

The total number of *HFE* variants detected to date is at least 37, of which 19 are missense[11,29] (see also The Human Gene Mutation Database). The most common mutation is *C282Y*. The cysteine at position 282 within the immunoglobulin domain constant region takes part in a critical disulfide bond. The C282Y mutation abolishes cell surface expression by preventing the association of the HFE gene product with beta-2 microglobulin.[40] The second most common mutation *H63D* results in measurable consequences on hepatic iron levels in mice[41] but does not cause HHC even in homozygous form in humans because of low penetrance and delayed action.[34,42,43] In combination with a *trans C282Y* mutation, however, *H63D* can cause HHC.[42] The compound heterozygosity for *C282Y* and *H63D* shows its effect on iron parameters at a level between *C282Y* homozygosity and *C282Y* heterozygosity.[34,44,45] The *C282Y* mutation causes HHC as a result of a deficiency of the HFE protein (loss-of-function) not by changing its function (gain-of-function) or cellular location. There is no sign of haploinsufficiency caused by heterozygous *C282Y* mutation.[43]

Toomajian and Kreitman[46] have conducted a comprehensive study of variation of the *HFE* gene on 60 chromosomes from three continents. They found a total of 41 polymorphic sites forming 18 distinct haplotypes in the 11,214-bp region including the flanking regions. Some of these polymorphic sites are in the 3' untranslated region of the gene and could conceivably affect mRNA stability or levels of protein translation. Other known polymorphisms in the 5' flanking region[47] or in intron 3[48] do not influence serum iron indices. Functionally important mutations of HFE and other iron-related genes have been listed in a recent publication.[29]

A number of genotyping methods have been used to type *HFE* variants. The most popular method is polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) analysis.[49] A multiplex PCR-RFLP method can type the two most common mutations in a single assay.[50] A comprehensive diagnostic assay for nonsynonymous changes in *HFE* and mutations in some of the other iron metabolism regulatory genes using PCR-sequence specific primer (PCR-SSP) method has been developed.[11] Kits for exhaustive *HFE* typings using reverse hybridization-based strip assay are available.[51] Other methods include real-time PCR,[52] SSCP,[53] heteroduplex analysis,[54] and denaturing HPLC.[55]

Table 2
Three most common *HFE* sequence variants

Amino acid change	Nucleotide change[a]	dbSNP no.	Recommended nomenclature[b]
H63D	3511C>G	rs1799945:C>G	NT_007592.13:g.3732C>G
S65C	3517A>T	rs1800730:A>T	NT_007592.13:g.3738A>T
C282Y	5473G>A	rs1800562:G>A	NT_007592.13:g.5694G>A

[a]Nucleotide numbering is from the translation initiation site. Reference sequence is NT 007592.13 (GI:29804415) in which the nucleotide 222 is the first nucleotide of the first codon ATG; [b]as recommended by Human Genome Variation Society (see Nomenclature for the Description of Sequence Variations). A fuller list of *HFE* mutations and their functional correlations are listed elsewhere.[11,29]

POPULATION FREQUENCIES

Population frequencies of *HFE* gene variants for geographic regions and ethnic groups have been presented in another

HuGE review[1] and in more recent reports.[34,56] In brief, the *C282Y* mutation is confined to populations of European origin and is most common in Northern Europe where the heterozygote frequency is 10% to 20%.[57]

DISEASE

Childhood leukemias are rare diseases. Only 1 in 100 new cancers is a childhood cancer and approximately 25% of childhood cancers are leukemias. Nearly 80% of childhood leukemias are acute lymphoblastic leukemia (ALL). Childhood ALL has been associated with prenatal exposure to ionizing radiation, certain chromosomal abnormalities, infections and an aberrant immune response to them, socioeconomic status, maternal and perinatal factors, various environmental exposures, and parental occupational history, but the actual causes are largely unknown.[58–60] Childhood leukemia is not inherited. It is more likely that genetic susceptibility increases the risk of an environmental exposure.[61] Molecular epidemiologic studies identified a number of genetic associations, mainly with genes encoding xenobiotic and DNA repair enzymes.[62,63] Another group of genes showing associations with childhood ALL are human leukocyte antigen (*HLA*) genes with a yet unknown biological mechanism.[64–66]

One of the most consistent findings in leukemia epidemiology is the increased male-to-female ratio.[60,67] Some genetic association studies have also found gender-specific associations[62,64,66] including the *HFE* association.[68] It appears that males may have a lower threshold for genetic factors to exert their effect. The multifactorial threshold model for pyloric stenosis is similar in that males have a lower threshold to be affected with the disease.[69,70] Why "maleness" lowers the liability threshold in leukemia is unknown but possible reasons include an epigenetic one as postulated for autism.[71]

ASSOCIATIONS

HFE is one of the molecules that participate in iron homeostasis. It has been postulated that its main role is in iron transport across the cell membrane including the regulation of absorption in the gastrointestinal tract.[72] Wild-type HFE reduces the affinity of transferrin receptor for transferrin-bound iron,[73] comigrates with it inside the cell,[74] and regulates cellular uptake of iron from transferrin within endocytic compartments.[75] When its expression is hampered, the interactions with transferrin receptor on the cell surface and with transferrin inside the cell do not occur and iron influx is increased. Despite these findings, it is possible that it has a more important role in controlling expression of hepcidin (encoded by *HAMP*), which has a regulatory role in downregulating the intestinal iron absorption, placental transport of iron, and the release of iron by macrophages.[9,16]

A twin study identified a considerable "additive" genetic component in body iron level regulation. Within that component, the share of *HFE* mutations was less than one would expect.[76] The small share of *HFE* variation in total heritability has been confirmed in a population-based study.[77] A study of sib-pairs homozygous for *C282Y* showed significant variation in iron overload between siblings.[3] These findings attribute a larger role to other genes involved in iron absorption, transport, and storage. Two such genes, *HAMP* and *HFE2*, have been shown to modify the expression of *C282Y* homozygosity in HHC.[13,15,17] The expression of *C282Y* mutation, in homozygous or heterozygous form, may require genetic modifiers and environmental interactions to have an effect on body iron content.

Although not usually causing HHC, heterozygosity for *C282Y* may also be relevant in disease susceptibility other than HHC. On average, 1 in 10 individuals in European populations may be heterozygous for the *C282Y* mutation.[1,34,49,56] This is a frightening frequency if carriers of this mutation are in any way vulnerable to any disease.

A study of 1058 heterozygotes ascertained from 202 pedigrees by family *HLA* typing revealed that serum iron and ferritin concentrations and transferrin saturation values generally overlapped with the normal range but were higher in 15% to 25% of heterozygotes.[78] Although mean transferrin saturation in *C282Y* heterozygotes is only slightly elevated, the magnitude of elevation was similar to that reported as a risk factor for cancer in cohort studies.[79]

The findings of the study by Bulaj et al.[78] corroborate with those of other family- or population-based studies. In most studies, up to 25% of heterozygotes have minor subclinical iron status changes.[31–34,80–88] In general, the population studies show a small but significant increase in transferrin saturation and a small but usually insignificant increase in serum ferritin. *HFE* heterozygosity has been confirmed as one of the genetic factors affecting body iron content also in a twin study.[76] A meta-analysis of 14 studies concluded that *C282Y* heterozygosity is associated with a 4-fold risk of increased iron stores (95% confidence interval = 2.9 to 5.8), although the reliability of this result was low due to heterogeneity.[44] In a minority of patients with HHC, heterozygosity for *C282Y* may even be the only mutation detectable out of the three major *HFE* mutations.[31] An animal study showed that *C282Y* heterozygosity is capable of increasing iron levels.[89] Another animal study noted the importance of genetic background in the expression of *HFE* mutations.[90] Ethnicity may be a modifier in association studies because of the variation in other genes involved in iron homeostasis. To date, only one study has suggested higher penetrance for *H63D* in Hispanics[5] but this finding needs replication. The overall conclusion is that heterozygosity for the *C282Y* mutation of *HFE* may increase serum iron levels in a subset of carriers. Similar to *C282Y* heterozygosity, a very mild effect of *S65C* mutation on iron overload has also been noted.[91] The associations described later are most likely the result of serum iron elevation in heterozygotes.

Association of iron levels with cancer

Increased risk for cancer in subjects with even moderately elevated serum iron levels has been shown repeatedly. Prospective cohort studies including between 6,000 and 174,000 sub-

jects have reported a link between indicators of high iron stores and increased relative risk for cancer in general.[79,92–97] These studies include the first and second National Health and Nutrition Examination Surveys. Additional case-control studies revealed the same link in colon[98] and liver cancer.[99,100] Some studies yielded negative results in gastric[101] and epithelial cancers.[102] The link between increased body iron and cancer was also suggested by the decreased cancer incidence in regular blood donors in Sweden.[103]

Increased intracellular iron can influence the process of carcinogenesis by catalyzing the formation of mutagenic hydroxyl radicals, by acting as an essential nutrient for proliferating neoplastic cells, or by its deleterious effects on the immune system.[104–106] One of the immune disturbances in iron overload is the higher average CD4:CD8 ratios,and this is not related to the mutations in *HFE* but directly to iron.[107] The evidence for a procarcinogenic role of iron is presented in Table 3.

As the major site of iron storage, the liver is most sensitive to iron overload. As a result, liver cancer risk secondary to cirrhosis is enormously increased in HHC and the risk is also increased in non-HHC iron overload (see Table 3). In a study of 230 patients and 230 controls with noniron-related chronic liver diseases, the increased risk for extra hepatic cancers in HHC showed no correlation with *HFE* genotype, indicating that it is iron itself but not *HFE* that confers risk for cancer.[130] HHC is not the only oxyradical overload disease. Another hereditary disease characterized by intracellular copper overload, Wilson disease (OMIM 277900), also shows increased long-term risk with internal malignancies including hepatoma.[131] Higher expression of biomarkers for oxidative stress and increased frequency of *P53* tumor suppressor gene have been observed in both oxyradical overload diseases.[132] More frequent spontaneous and radiation-induced chromosomal damage in HHC[133] may be an important mechanism for cancer development in iron overload. Although not a uniform finding, several studies reported an increased risk for extrahepatic cancer in HHC.[124,130]

Table 3
Evidence suggesting a procarcinogenic role for iron

Finding or observation	References
Iron-induced oxidative DNA damage	104,106,108–110
Iron-induced abnormalities of immune function	107,111,112
Increased susceptibility to viral infections	113,114
Iron as an essential nutrient for proliferating neoplastic cells	115–118
Animal experiments showing procarcinogenic effect of iron	105,116,117,119–121
Increased liver cancer risk in HHC	122–126
Increased liver cancer risk in non-HHC iron overload	105,127,128
Correlation between serum iron levels and cancer risk	79,92–100,129
Correlation between regular blood donation and reduced cancer risk	103

Association of *HFE* with leukemia and lymphoma

An earlier study found an increased risk for cancer in obligatory heterozygotes for the putative HHC gene.[134] The association with hematologic malignancies was restricted to males. After the discovery of *HFE* as the HHC gene,[2] a number of studies have investigated *C282Y* and *H63D* mutations in different cancers. The first ones were conducted by Beckman et al.[25–27] who found an increased frequency of *C282Y* mutation in multiple myeloma, breast, colorectal, and liver cancers, but only in interaction with the *S142G* (*g.424A>G*) polymorphism of *TFRC*. Since then, *C282Y* associations have been reported in colon[135] and breast cancer[136] and an *H63D* association in malignant glioma.[137] Two studies did not find any increase in *C282Y* frequency in colon cancer.[138,139] One study investigated the *HFE* mutations in a series of cancers and did not find a generally increased frequency.[140] We have recently determined the *C282Y* frequency in 147 cases with human immunodeficiency virus (HIV)–induced Kaposi sarcoma and their HIV and 147 human Kaposi sarcoma herpes virus (KSHV) double-positive matched controls all from the Multicenter AIDS Cohort Study (MACS).[141] We did that because of the suggestion that iron is involved in the pathogenesis of classic Kaposi sarcoma.[142] The matched pair analysis by conditional logistic regression yielded an odds ratio of 5.4 (95% CI = 1.8 to 16.4; $P = 0.0009$). The mutation frequency was 14.5% in cases (all heterozygous) and 3.0% in matched-controls. It is unknown whether *C282Y* is associated with cancers because of its effect on body iron content or linkage disequilibrium (LD) with another gene. The Kaposi sarcoma study also investigated the *HLA* complex and endothelin-1 gene (*EDN1*) on either side of *HFE* (M.T. Dorak et al., manuscript in preparation, 2005). The *C282Y* association was independent of the other associations found with *EDN1* and *HLA* genes.

HFE associations have been sought also hematologic malignancies (Table 4). We reported the *C282Y* frequencies in childhood acute lymphoblastic leukemia (ALL).[68] In a case-control study of Welsh and Scottish patient groups, there was an increase in *C282Y* mutation frequency compared to newborns from respective newborn controls but in males only. The association mainly concerned heterozygosity for *C282Y*. *H63D* was examined only in the larger Scottish group and did not seem to contribute to leukemia susceptibility. Recent detailed work ruled out LD with *EDN1* and several *HLA* complex loci as the reason for this association.[143]

There is one other published report on the *C282Y* mutation in childhood leukemia from Finland.[148] In a study of 232 mainly adult patients with various hematologic malignancies, 32 patients with childhood ALL (14 boys) did not have an increased frequency of *C282Y*. The Finnish study did not find an increased frequency in any of the subsets ($n = 15$ to 53). In another study of 36 Spanish patients with adult acute myeloid leukemia and 108 controls, the frequencies for *C282Y* and *H63D* were not different between cases and controls.[145] Both

Table 4
Associations of hematologic malignancies with *C282Y*

Disease	Case/control numbers	Controls	*C282Y* frequencies (%)[a]	Odds ratio (95% CI; *P*)	References
Childhood ALL (Wales)	117/415	Local newborns	23.4 vs. 12.3[b]	2.19 (1.14 to 4.18; $P = 0.03$)	68
Childhood ALL (Scotland)	135/238	Local newborns	34.7 vs. 15.1[c]	2.98 (1.65 to 5.39; $P = 0.0004$)	68
Adult Hodgkin's disease (Wales)	121/10556	Local blood donors	11.7 vs. 15.8[d]	NS	144
Adult Acute Myeloid Leukemia (Spain)	36/108	Local blood donors	8.3 vs. 7.4	NS	145
Myelodysplastic syndrome (Hungary)	50/80	Local blood donors	10.0 vs. 5.0	2.11 (0.54 to 8.27; NS)[e]	146
Myelodysplastic syndrome (Greece)	54/264	Local blood donors	0.0 vs. 0.0	NS[f]	147
Hematologic malignancies (Finland)	232/128	Local medical students	8.2 vs. 10.2	NS	148
Hematologic malignancies (AL, USA)	52/318	Local population controls	0.04–0.21 vs. 0.09	0.4 to 2.8 (NS)[g]	140
Hematologic malignancies (transplant patients) (TN, USA)	129/118	Local controls	17.0 vs. 12.7	1.41 (0.69 to 2.87; NS)[h]	136

[a]All frequencies are marker frequencies (proportion of individuals positive for *C282Y*); [b]male patients only (n = 64); [c]male patients only (n = 75); [d]control frequency is from ref. 34; [e]this study reported a significant increase in combined *HFE* mutation (*C282Y* and/or *H63D*) frequency in patients (52.0% vs. 31.3%; OR = 2.38, 95% CI = 1.15 to 4.94, $P = 0.03$); [f]comparison of *H63D* mutation frequencies between cases and controls yielded an OR of 1.87 (95% CI = 0.95 to 3.68, Fisher's $P = 0.08$-our calculation); [g]ORs vary for each subset with n = 5 to 13; [h]our calculation.

these studies appear to have shown negative results but obviously they were underpowered to detect significant differences. Notably, the two studies that have shown an association in childhood leukemia are British studies and to what extent this finding can be generalized to other populations is currently unknown.

Other studies of *HFE* associations in hematologic malignancies included our own adult Hodgkin disease case-control study,[144] which revealed no association; and a myelodysplastic syndrome study in Hungary with a positive association,[146] which could not be replicated in Greece.[147] In the breast cancer study performed in Tennessee, the cases included patients with hematologic malignancies transplanted in the same center.[136] The *C282Y* frequency in this subset was 17.0% ($n = 129$) compared with local (12.7%, $n = 118$) and national (12.4%, $n = 2016$) mutation frequencies, which appears to be increased.

All *HFE*-cancer association studies reported to date are case-control studies that have recognized limitations. Chance associations cannot be ruled out—even with replication—until functional studies identify the biological mechanism of the reported associations.

Possible mechanism of leukemia association

In other cancers associated with *C282Y*, statistical interactions with a *TFRC* allele,[25–27] increased iron intake in diet and older age,[135] and correlation between *C282Y* gene dosage and body iron stores in breast cancer[136] strongly argue that the mechanism of the *HFE* associations with cancer is related to iron. Thus, molecular *HFE* association studies seem to complement the effect of elevated iron on cancer risk. The question that whether the same risk applies to a childhood cancer has not been tested experimentally.

HFE is expressed by lymphoid as well as myeloid cells. In a B-lymphoid cell line homozygous for *C282Y* and analyzed in detail, iron uptake is increased and cell sensitivity to oxidative stress is enhanced.[149] This sensitivity to oxidative stress is crucial in iron-induced carcinogenesis.[104–106,108–110] Chronically increased oxidative stress from elevated levels of iron in the body may increase radiation sensitivity by decreasing cellular oxygen radical scavenging capability. Low-level radiation sensitization by iron, which can occur in lymphocytes, has been proposed to increase cancer susceptibility,[150,151] and heterozygosity for *HFE* mutations has been emphasized as a risk factor.[152,153] Given the higher sensitivity to environmental exposures during early development, *C282Y* heterozygote fetuses, especially if their mothers are the origin of their mutation, may be subject to higher intracellular iron levels in their lymphoid cells. This may have a promoter effect if a lymphoid cell has leukemic transformation spontaneously or due to environmental exposure. Unlike adult cancers, no link has been investigated between body iron stores and childhood cancer, but in neuroblastoma, Hodgkin disease, and ALL, an unfavorable effect of increased iron stores has been shown on survival.[154–156] There is a putative link between viral infection and childhood ALL.[58,157] Elevated iron levels in lymphoid cells may be relevant in this context because iron favors viral infections in animals.[113,114] Damage caused by iron overload in internal organs takes years, and is sex- and age-dependent. The proposed mechanism for childhood ALL entails increased intracellular iron levels in lymphoid cells during development. If iron contributes to childhood ALL susceptibility, other genes with roles in iron metabolism (Table 1) are expected to show associations. This can be investigated in incident case studies of childhood leukemia and other cancers to separate the effects of iron from a genetic association secondary to LD with *C282Y* mutation.

At present, all cancer and leukemia associations with *HFE* are no more than statistical associations. Assuming they are real, an alternative mechanism to be explored is other genes around the *HFE* locus. Several candidates already exist.[144]

Among those, *EDN1* is a strong one to be responsible for the *C282Y* association through LD. Although our preliminary study of *EDN1* in childhood ALL showed a weak but independent association with no LD with *C282Y*,[143] a more comprehensive study is required to rule out the involvement of neighboring genes and even other variants of *HFE* in *C282Y* association.

Association of *HFE* with nonmalignant diseases

Besides HHC, associations have suggested that the *HFE* mutations may also be involved in the development of other nonmalignant diseases. These include cardiovascular diseases, diabetes, arthritis, neurodegenerative disorders, and alcoholic liver disease.[45,158] Most of these associations, however, have been inconsistent. A list of conditions showing genetic associations with *HFE* is being compiled at the NCBI Genetic Associations Database.

It has to be underlined that none of the disease associations suggests a uniformly deleterious effect of *C282Y* mutation. If this was the case, one would expect a negative association between *C282Y* and longevity. Despite an early suggestion, latest studies conclusively ruled out an age-related decline in *C282Y* frequency.[56,159] More comprehensive studies taking into account genetic and environmental interactions are needed to conclude whether a subgroup of *HFE* mutation carriers has higher rates of disease and what additional factors identify that subset.

INTERACTIONS

Only one gene-gene interaction, between *HFE* and *TFRC* in multiple myeloma,[25] and no gene-environment interaction has been investigated in hematological malignancies. In iron overload, however, a number of factors in addition to *HFE* mutations affect the severity. In the most extreme example of *Hfe* knockout mice, the strain of mice determines the amount of iron in the liver.[160] *HAMP*[17] and *TFRC* gene polymorphisms,[25–27] mitochondrial DNA mutations,[161] parent-of-origin,[78] and environmental factors (including pregnancy, regular blood loss, iron intake, hepatitis B and C, and alcohol) have been suggested to interact with *C282Y* in its associations with diseases or in its effect on biochemical parameters of iron stores.[1,6,9,76,162–164] The *P570S* polymorphism of the transferrin gene shows an epistatic interaction with *C282Y* as a risk marker for Alzheimer disease.[24] This variant of *TF* has not been examined in biochemical iron overload states.

Phenotypic expression of HHC is affected by the presence or absence of the telomeric *HLA* ancestral haplotype characterized by *HLA-A*03*, *D6S265-1*, and *D6S105-8*.[165,166] Patients bearing this haplotype tend to have more severe forms of HHC, and this effect is dependent on gene dosage.[81,167,168] However, this has not been a universal finding.[169] Because of the effects of the ancestral haplotype on disease phenotype, *HFE* association studies in other diseases need to cover the area between *HLA-A* and *HFE*. This may have some bearing on the different results for the same genetic association in different populations.

GAPS AND RESEARCH PRIORITIES

Leukemia and lymphoma associations with *HFE* have not been sufficiently studied. Available studies are relatively small case-control studies.

Definitive studies are needed

Given the population frequency of common *HFE* variants and potential implications of any disease association on public health, there is need for a definitive study on *HFE* associations in hematopoietic cancers and especially for their mechanisms. Despite the rarity of childhood leukemias, Children's Oncology Group in USA and United Kingdom Childhood Cancer Study Group have been collecting large numbers of samples through nationwide recruitments. However, it is also important to perform association studies in other ethnic groups. Given the strong effect modification by sex, even comparison of male patients with female patients (without controls) may provide clues whether the male-specific *HFE* association can be confirmed in large prospective incident case studies.

Other iron-related genes need to be tested

The influence of *HFE* on body iron stores is small. If an iron-related mechanism is operating, variants of other genes taking part in iron homeostasis should also show associations. This is particularly important in geographic regions where *HFE* variants have small frequencies.

Possible gene-gene interactions should be addressed

An interaction between *HFE* and another gene in the region between *HFE* and *HLA-A* may explain the ancestral haplotype effect on HHC phenotype observed in some studies. Similarly, the *HFE* gene itself should be thoroughly examined especially in its regulatory regions. Interactions with the known polymorphisms of other genes such as *TFRC* (*424A*), *HAMP* (*R59G*, *G71D*, or *R56X*), and *HFE2/HJV* (*S105L*, *E302K*, *N372D*, *R335Q*, *L101P*, *G320V*) that have an effect on iron status are important ones. No cancer association studies with *HFE* concurrently examined the genes encoding antioxidant enzymes. Cellular antioxidant defense mechanisms against prooxidant states include enzymes such as superoxide dismutase, catalase, and glutathione systems.[104,170] Given the antipromoter and anticarcinogen activity of antioxidant defenses,[171,172] deleterious effect of iron would be greater in states of reduced antioxidant reserve. The genes for these enzymes have known functional polymorphisms[170] and these may also interact with *HFE* mutations to increase susceptibility to leukemia.

Gene-environment interactions require attention

Exposure to environmental iron during pregnancy or early childhood may interact with *HFE* variants in determination of leukemia susceptibility. A gene-environment interaction may also be shown with routine iron supplementation during pregnancy except when indicated for iron deficiency. In light of other genetic effect modifiers of common exposures,[173] exam-

ination of an interaction between maternal iron intake and the presence of *C282Y* in the offspring may be a worthwhile effort.

Parent-of-origin effect has not been studied

The association with childhood ALL may have to do with intrauterine environment if it is due to the generally increased sensitivity of developing child (from fetus to prepuberty) to environmental assaults.[174] It is a possibility that the association may be restricted to *C282Y* carriers who have inherited it from their mothers and whose intrauterine environment had elevated levels of iron because of heterozygosity in mothers.

A large case-control study that would carefully construct the functionally relevant haplotypic variants of the *HFE* gene, examine selected other genes involved in iron metabolism with incorporation of appropriate questionnaire data on iron supplementation and dietary habits is going to be an appropriate first step to fill the gap of knowledge outlined above, especially when conducted where the evidence appears to be strongest for childhood leukemia association. A prospective family-based association study can achieve the same purpose while providing additional information on the question of parent-of-origin effect.

CONCLUSIONS

The male-specific association of *C282Y* with childhood ALL in two populations seems to have generated useful hypotheses that can be tested. Currently available evidence suggests that this association with leukemia susceptibility arises from its effect on body iron levels. The sex-dependent penetrance of *C282Y* is age-dependent and cannot explain the male-specificity of the *C282Y* association in childhood. The presence of other male-specific genetic associations with childhood leukemia brings about the possibility of an additive role for these susceptibility markers over and above the risk conferred by "maleness" in a multifactorial threshold model. While what makes maleness a risk factor in genetic terms is studied, the *HFE*-associated susceptibility to childhood leukemia will have to be elaborated by extending the association studies to other iron-related functional genes and by taking into account gene and environment interactions. The *C282Y* association in leukemia and other cancers may highlight the need to focus on the known connection between iron and cancer in which *HFE* plays only a limited role. The very high frequency of *C282Y* in European populations does not mean that we are dealing with a problem restricted to Europe and America. If this association is due to elevated iron levels at biochemical level, the implications on the risks being inflicted by food-iron fortification programs, uncontrolled supplemental iron intake, and routine iron prescription in pregnancy will be worldwide.

Electronic resources

The following electronic resources were consulted for this review: Gene Tests (http://www.genetests.org), Kowdley et al. provide a regularly updated review of HHC and its genetics; NCBI Entrez Gene (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=gene) replacing NCBI LocusLink; NCBI OMIM, Online Mendelian Inheritance in Man (http://www.ncbi.nlm.nih.gov/entrez/query.fcgi?db=omim); NCBI Genetic Associations Database (http://geneticassociationdb.nih.gov); The Human Gene Mutation Database (Cardiff, UK) (http://archive.uwcm.ac.uk/uwcm/mg/search/119309.html); and Nomenclature for the Description of Sequence Variations (Human Genome Variation Society) (http://www.genomic.unimelb.edu.au/mdi/mutnomen).[12–30,101–130]

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